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REVIEW ARTICLE

Streptococcus mutans and cardiovascular diseases

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Summary *Streptococcus mutans*, a pathogen of dental caries, is known to be associated with bacteremia and infective endocarditis (IE). The bacterium has been classified into four serotypes, *c*, *e*, *f*, and *k*, based on the chemical composition of the serotype-specific rhamnose-glucose polymers. Serotype *k*, recently designated and initially found in blood isolates, features a drastic reduction of glucose side chains attached to the rhamnose backbone. Glucosyltransferases (GTFs), protein antigen (PA), and glucan-binding proteins (Gbps) are major surface protein antigens of *S. mutans*, and *in vitro* analyses using isogenic mutants without those cell surface proteins showed that a PA-defective mutant had the least susceptibility to phagocytosis. Further, rat experiments demonstrated that infection with such defective mutants resulted in a longer duration of bacteremia, while *S. mutans* strains without GTFs were isolated from the extirpated heart valve of an IE patient. These results imply that some variation of cell surface components is correlated to the virulence of IE caused by *S. mutans*. In addition, *S. mutans* DNA has been frequently identified in cardiovascular specimens at a higher ratio than other periodontal bacteria, indicating its possible involvement in various types of cardiovascular diseases beside bacteremia and IE.

Contents

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Table 1 Major surface protein antigens of *S. mutans* and their coding genes

Protein antigen	Gene name	GenBank locus tag	Definition	Gene length	Estimated molecular weight (Da)
GTFB	*gtfB*	SMU.1004	Glucosyltransferase-I	4431	165,733
GTFC	*gtfC*	SMU.1005	Glucosyltransferase-SI	4368	162,970
GTFD	*gtfD*	SMU.910	Glucosyltransferase-S	4389	163,393
PA	*pac*	SMU.610	Cell surface antigen	4689	169,997
GbpA	*gbpA*	SMU.2112c	Glucan-binding protein	1698	63,118
GbpB	*gbpB*	SMU.22	Glucan-binding protein B	1296	44,608
GbpC	*gbpC*	SMU.1396c	Glucan-binding protein C	1752	63,340
GbpD	*gbpD*	SMU.772	Bifunctional glucan-binding protein D and lipase	2181	79,780

The description of each protein antigen and its coding gene in the strain UA159 was extracted from the ORALGEN database (http://www.oralgen.lanl.gov/).

1. *Streptococcus mutans* and dental caries

Streptococcus mutans, a Gram-positive facultative anaerobic bacterium, is generally known to be a pathogen of dental caries [1], and its surface protein antigens have been investigated to clarify their role as virulence factors. Glucosyltransferases (GTFs), protein antigen (PA), and glucan-binding proteins (Gbps) are known to be major surface protein antigens of *S. mutans* (Table 1), with three types of GTFs (GTFB/GTFC/GTFD) shown to be involved in sucrose-dependent adhesion to tooth surfaces [2—4]. GTFB and GTFD are reported to produce insoluble and soluble glucans, respectively, from sucrose, while GTFC is involved with the production of both types of glucan. In contrast, PA [5] also referred to SpaP [6], antigen I/II [7], antigen B [8], SR [9], IF [10], P1 [11], and MSL-1 [12], has been shown to be correlated with sucrose-independent initial adhesion to tooth surfaces. Four types of Gbps (GbpA/GbpB/GbpC/GbpD) of *S. mutans* are also regarded as virulence factors for dental caries, due to their glucan-binding properties [13—16]. *In vitro* analyses to elucidate the mechanism of dental caries have been carried out using isogenic mutants defective of target proteins, which were constructed by inactivation of the genes encoding each protein antigen [17—19]. Animal experiments using specific pathogen-free rats orally infected with isogenic mutants and their parent strain showed a significant involvement of these protein antigens with dental caries [20—22].

2. Serotypes of *S. mutans*

Until recently, *S. mutans* was classified into three serotypes, *c*, *e*, and *f*, based on the chemical composition of serotype-specific rhamnose-glucose polymers (RGPs), which are composed of a backbone of rhamnose and side chains of α1,2-(serotype *c*), β1,2-(serotype *e*) and α1,3-(serotype *f*) glucosidic residues (Fig. 1) [23]. Genes involved in the biosynthesis of RGPs have been cloned and sequenced, of which four *rml* genes (*rmlA-D*) are known to be related to the synthesis of dTDP-L-rhamnose, a rhamnose backbone unit [24,25]. On the other hand, *gluA* is known to be involved in the production of the immediate precursor of the glucose side chain [26], and the six-gene operon (*rgpA-rgpF*) and *rgpG*, which are required for the synthesis of RGPs, have also been cloned and sequenced [27,28]. In addition, genes located just downstream of the *rgpA-rgpF* operon have been sequenced. This region was shown to be highly diverse among the serotypes and a PCR method for specification of each serotype was constructed using the nucleotide alignments specific for each serotype in the region [29].

Several non-*c*/*e*/*f* *S. mutans* strains have been described in previous reports. However, only a single study has provided information regarding the sugar composition of RGPs. Fig. 2 shows the sugar composition of purified serotype-specific polysaccharides analyzed by high-liquid performance chromatography, which identified a drastic reduction in the amount of glucose in a non-*c*/*e*/*f* strain (TW295) isolated from human blood as compared to the reference strain (MT8148) [30]. *S. mutans* serotype-specific antisera are generally produced by intramuscular immunization of whole cells in rabbits, however, antisera for non-*c*/*e*/*f* strains cannot be produced by the conventional method. A modification of the immunization protocol by repeated intravenous injections of whole cells resulted in successful generation of antisera specific against these strains, which led to the recent designation of novel serotype *k* [31]. Thereafter, non-*c*/*e*/*f* strains from saliva and dental plaque samples taken from Japanese children were classified as serotype *k* when analyzed by an immunodiffusion technique using serotype *k*-specific antiserum [32].

The prevalence rates of serotypes of *S. mutans* presented in past articles are summarized in Table 2 [29,31—43]. There

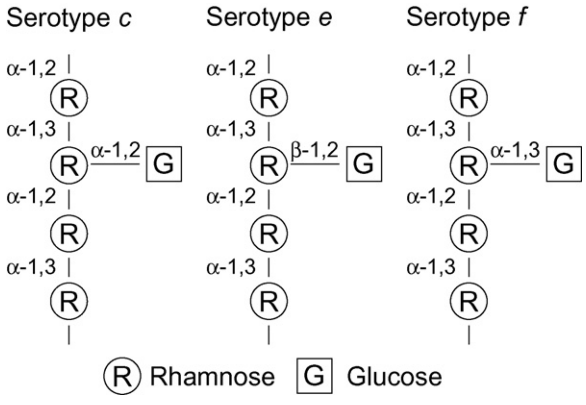

Figure 1 Illustration of chemical composition of serotype-specific polysaccharides in *S. mutans*.

Table 2 Prevalence of *S. mutans* serotypes reported in previous reports

Authors[a]	Country	Publication year	Methods	Total subjects	Total strains	Serotype prevalence (%)			
						c	*e*	*f*	Non-*c*/*e*/*f*
Bratthall [33]	England and others[b]	1970	Immunodiffusion	—	56	89.3	8.9	1.8 (non-*c*/*e*)[c]	
Perch et al. [34]	Denmark	1974	Fluorescent antibody	—	171	83.0	7.0	5.3	4.7[d]
Huis in't Veld et al. [35]	Netherlands	1979	Ring precipitation	—	100	77.0	13.0	10.0	0
Hamada et al. [36]	Japan	1980	Immunodiffusion	—	997	68.9	13.3	17.8	0
Fitzgerald et al. [37]	USA	1983	Fluorescent antibody	—	96	67.7	11.5	4.2	16.7
Holbrook and Beighton [38]	Iceland	1986	Immunodiffusion	63	78	73.1	24.4	2.6 (non-*c*/*e*)[c]	
Beighton et al. [39]	UK	1987	Immunodiffusion	59	69	72.5	26.1	1.4 (non-*c*/*e*)[c]	
Batoni et al. [40]	Italy	1993	ELISA	—	136	78.7 (*c* and *f*)	16.2	—	5.1
Kozai et al. [41]	Japan	1999	Immunodiffusion	68	114	84.2	15.8	0	0
Hirasawa and Takada [42]	Japan	2003	Immunodiffusion	85	93	64.5	24.7	1.1	9.7
Shibata et al. [29]	Japan	2003	PCR	211	—	84.4	13.9	1.9	0.5
Nakano et al. [31][e]	Japan	2004	Immunodiffusion	—	1326	85.3	12.3	2.4	0
Nakano et al. [31][f]	Japan	2004	Immunodiffusion	100	100	78.0	17.0	3.0	2.0 (*k*)
Nakano et al. [31][g]	Japan	2004	Immunodiffusion	50	2500	70.8	22.0	5.2	2.0 (*k*)
Nakano et al. [43][h]	Japan	2004	PCR	137	—	72.8	21.9	4.1	1.2 (*k*)

[a]Numbers in parentheses indicate reference numbers. [b]Sweden, Switzerland, and USA. [c]OMZ175, which was classified as *f* in a following study. [d]May include both non-*c*/*e*/*f* *S. mutans* and *S. sobrinus* strains. [e,f,g,h]Year of isolation; [e]1982—1990, [f]2002, [g]2003 and [h]2005—2006.

are several methods used to determine serotypes, among which an immunodiffusion method using cell extract antigens and serotype-specific antisera is commonly utilized. Further, PCR methods that identify serotype using bacterial DNA have been recently developed [29,32]. With those, isolation of strains is not necessary for serotype determination, as the serotypes of *S. mutans* in saliva, dental plaque, and other tissues can be easily specified. In addition, detection sensitivity has been shown to be higher with such a PCR method as compared to a conventional immunodiffusion technique [32].

The detection rate of serotype *c* strains has been reported to be the highest (ranging from 64.5 to 89.3%), followed by serotype *e* (from 7.0 to 26.1%) (Table 2) [29,31—43]. In contrast, the detection rate of serotype *f* is estimated to be less than 5%, based on the results of previous studies, though a higher frequency was reported in early studies. Prior to the designation of serotype *k*, there were several reports of the existence of non-*c*/*e*/*f* serotype in samples from subjects in European countries, as well as from the United States and Japan. It is not possible to speculate whether those strains belong to serotype *k*, since no description of the sugar composition of their serotype-specific RGPs was provided. The distribution of serotype *k* organisms in the oral cavity has been estimated to range from 2 to 5%, based on studies held in Japan [31,32]. Recently, Waterhouse and Russell [44] designated a strain originally isolated in the UK in the 1990s (strain 21) as serotype *k*. In addition, several non-*c*/*e*/*f* *S. mutans* strains isolated in Finland in the early 1990s were recently classified as serotype *k* [45]. These findings indicate that serotype *k* strains exist outside of Japan, including some previously reported non-*c*/*e*/*f* strains.

It would be interesting to understand the evolution of each serotype of *S. mutans*. A recently completed multilocus sequence typing scheme for *S. mutans* showed that serotype *c* strains were widely distributed in the dendrograms, and that serotypes *e*, *f*, and *k* strains were differentiated into clonal complexes [45]. Therefore, the ancestral strain of *S. mutans* may be serotype *c*, with the other serotypes continuously branched from serotype *c* groups.

3. Serotype *k* strains

An analysis of genes involved in the biosynthesis of serotype-specific RGPs found that the serotype *k*-specific nucleotide alignment was present in the 5′ region of the *rgpF* gene (350 bp from the initial sequence), thus the PCR method used for identification of serotype *k* was constructed using the alignment of that region [32]. It is speculated that an alteration of the *rgpF* alignment in serotype *k* strains as compared to other serotypes may cause the drastic reduction of glucose side chains evident in those RGPs. However, the serotype of mutants constructed from serotype *c* strains, in which the *rgpF* sequence was replaced with that of serotype *k* strains, was shown to be serotype *c*, indicating that an alteration of *rgpF* is not associated with a reduction of glucose side chains in RGPs [46]. On the other hand, mRNA expression of *rgpE* was found to be diminished in serotype *k* strains in a following study, which was estimated to be the major cause of the glucose side chain reduction commonly identified in serotype *k* strains [46].

Isolation and designation of serotype *k* *S. mutans* strains have been reported in several studies, with the cariogenicity

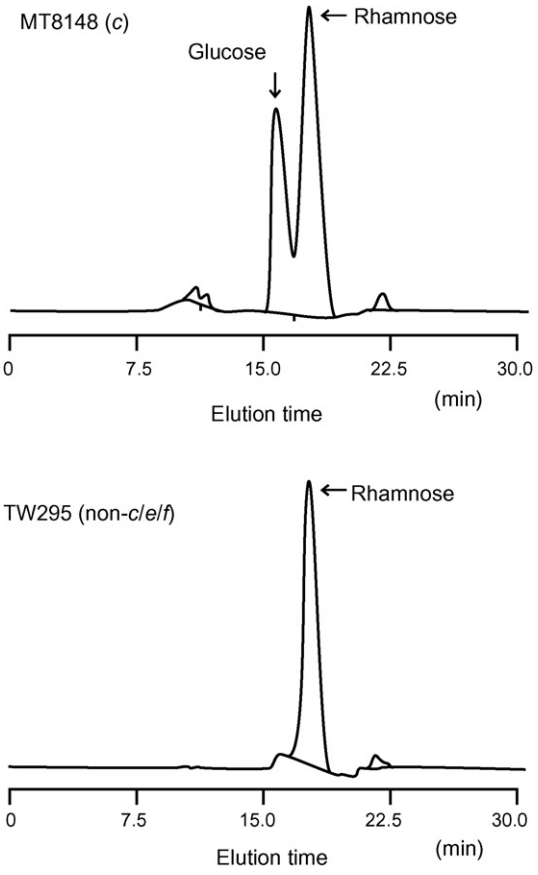

Figure 2 Sugar composition analysis of purified serotype-specific polysaccharides of *S. mutans* by high-performance liquid chromatography.

of a single strain (TW871) thoroughly investigated in one of those [47]. That strain exhibited lower caries-inducing properties *in vitro* as well as in a rat model of dental caries due to attenuation of its GbpC expression. In addition, GbpA expression was not detected in that strain due to a defect of the *gbpA* gene [47]. When PA expression was analyzed in various clinical isolates of *S. mutans*, defects of PA expression were frequently identified in serotype *k* strains [18]. These results indicate that alteration of cell surface proteins is another characteristic of serotype *k* strains.

Since serotype *k* organisms feature a drastic reduction of glucose side chains in the serotype-specific RGPs, it is important to analyze the association of that glucose side chain defect with virulence. Previous *in vitro* and *in vivo* assays using glucose side chain-defective isogenic mutants indicated that the glucose side chain itself is associated with cariogenicity, though to a lesser extent than the other major surface proteins of *S. mutans* [48]. In addition, glucose side chain-defective mutants were shown to be significantly less susceptible to phagocytosis by polymorphonuclear leukocytes, indicating that serotype *k* strains may cause a longer duration of bacteremia as compared to other serotypes [31].

4. *S. mutans* and bacteremia

Dissemination of oral bacteria into the bloodstream is known to be induced by professional dental treatment and daily oral care practices, such as tooth brushing and flossing, and even food chewing [49]. Also, serotype-specific RGPs were shown to contribute to the resistance to phagocytosis by human polymorphonuclear leukocytes [50]. Previously, the phagocytosis susceptibility of two *S. mutans* strains isolated from patients with bacteremia (strains TW295 and MH1) was evaluated following incubation of the tested bacteria (5.0×10^7 CFU) with human peripheral blood (500 μL) at 37 °C for 10 min. The results showed that both had lower phagocytosis rates as compared to the reference strain MT8148 [31,51].

The phagocytosis susceptibility of GTF-, PA-, Gbp-defective isogenic mutant strains was also analyzed using the same method, which showed that the PA-defective mutant was the least susceptible to phagocytosis, followed by a GbpC-defective strain (Fig. 3) [18,19]. In a rat bacteremia model, infection with a PA-defective mutant induced a significantly longer duration of bacteremia and the inflammation marker reached significantly higher levels than in rats infected with the parent strain [18]. Since this phenomenon was also observed with a GbpC-defective mutant [52] and GbpC is known to have a high homology with PA [15], PA and the related antigen are possibly associated with the virulence of bacteremia caused by *S. mutans*.

PA was also shown to be involved with the initial attachment of cells to tooth surfaces, and its deficiency was found to reduce cariogenicity in both *in vitro* and animal model experiments [21,53]. On the other hand, PA was reported to be immunodominant toward *S. mutans* antigens when serum antibody responses to systemic infection of oral streptococci were analyzed in humans [54]. Thus, strains with a deficiency of PA expression are not only less cariogenic, but also have fewer antigenic properties. Therefore, it is reasonable to speculate that strains without PA expression can survive in the bloodstream for a long duration, causing the persistence of bacteremia.

Biofilm regulatory protein A (BrpA) is known to regulate biofilm formation, while inactivation of the *brpA* gene encoding BrpA was shown to cause its long chain formation [55,56]. Further, a BrpA-defective mutant was less susceptible to

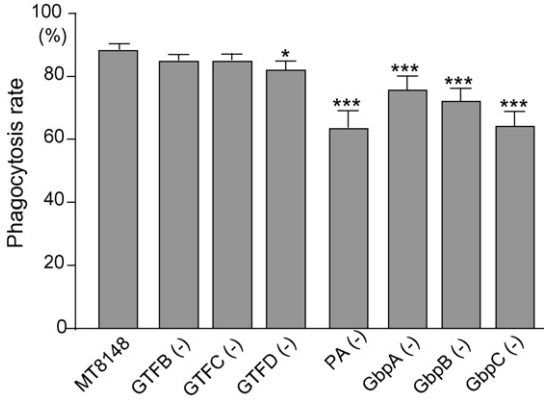

Figure 3 Phagocytosis susceptibility of *S. mutans* MT8148 and isogenic mutants defective of major surface proteins. Fisher's PLSD analysis (*$P < 0.05$ and ***$P < 0.001$ against MT8148).

phagocytosis by polymorphonuclear leukocytes and increased platelet aggregation properties as compared to its parent strain [57]. It is possible that other unknown proteins related to the virulence of bacteremia caused by *S. mutans* may be found in future studies.

5. *S. mutans* and infective endocarditis

The incidence of infective endocarditis (IE) was calculated in a review of 26 studies describing 3784 episodes of IE that occurred between 1993 and 2003, and found to be 3.6 per 100,000 per year (range 0.3—22.4) [58]. According to a review of 848 IE cases in Japan performed recently, the most common type of microorganisms isolated from patients was streptococcus species (49.5%), followed by staphylococcus species (31.7%) [59]. Further, the most frequently detected streptococcus species in patients with streptococcal valvular diseases is known to be *Streptococcus sanguinis*, while *S. mutans* was reported to be recovered from approximately 15% [60].

Table 3 summarizes a number of case reports of IE caused by *S. mutans*. IE is known to be divided into acute and subacute forms, and the bacterium is generally isolated in subacute cases, in which fever, chills, sweats, anorexia, weight loss, and malaise are common symptoms [61]. *S. mutans* is detected in blood culture examinations in most cases [62—70], though one patient with a negative blood culture result on admission had *S. mutans* detected at the second examination conducted 12 h later [66]. In two other case reports, *S. mutans* was specified with a molecular method using broad-range PCR and sequencing techniques [69,70]. Oral conditions suspected to induce IE have also been discussed in several reports [62—70]. However, the associations of those conditions with the onset of IE remain to be elucidated with a scientific approach in further studies [71].

As for the pathogenesis of IE, its primary occurrence results from bacterial adherence to damaged valves that produces vegetation [58]. Thus, animal models of IE require artificial damage to the heart valve by a catheter in the location of vegetation formation. Several reports have analyzed the contribution of the major cell surface structures of *S. mutans* to the onset of IE using animal models. Sucrose-derived exopolysaccharide was shown to contribute to infectivity in endocarditis caused by an *S. mutans* isogenic mutant strain with defects of all three types of GTFs that was generated from the blood isolate strain V403 [72]. However, *S. mutans* strains with a lack of expression of all three types of GTFs have also been isolated from infected heart valve tissues [70], indicating that GTFs do not directly contribute to the virulence of IE and that such defects may enhance the virulence of *S. mutans* in IE. This speculation is supported by the results of an animal experiment that demonstrated that the survival rate of mice infected with an isogenic mutant strain lacking all three types of GTFs was significantly lower than that of those infected with the parent strain as well as the uninfected control group [73].

Another animal experiment found that PA was not important for the onset of IE, since a PA-defective mutant showed similar adhesion properties to vegetation as the parent strain [74]. In contrast, RGPs were shown to contribute to the virulence of IE based on their properties of adhesion to collagen type I and laminin [75]. The lipoprotein receptor antigen I (LraI) was also shown to be important for the development of IE [76]. In addition, the Cnm protein, reported to function as a collagen-binding adhesin, was found to have an association with the initial bacterial attachment to blood vessels [77], and the rates of *cnm*-positive strains among serotype *f* and *k* strains were shown to be significantly higher than those of other serotypes [45]. Further, fibronectin-binding protein, considered to be involved in bacterial binding to extracellular matrix components such as fibrin, platelets and fibronectin, was proposed to be one of the crucial factors for IE caused by *S. mutans* [78]. As for platelet aggregation, serotype-specific RGPs were shown to possess properties that trigger platelet aggregation by direct binding and activation [79].

A blood culture examination using samples from possible IE patients is regarded as an important diagnostic tool, while molecular methods have also been recommended for diagnosis of IE [80]. Broad-range PCR and sequencing techniques, in which amplification and nucleotide determination of 16S rRNA gene fragments using primers targeting the sequence common in eubacterial species, are widely used to identify bacterial species [81]. Recently, molecular analyses of extirpated heart valve specimens from eight subacute IE cases were performed, and the results showed that seven specimens contained *S. mutans* DNA, of which five were positive for serotype *k* [82]. However, since the number of specimens was quite low, whether serotype *k S. mutans* was incidentally detected due to dissemination from the oral cavity or is an actual pathogen in each of the cases of IE remains to be elucidated. Nevertheless, it is possible to speculate that serotype *k S. mutans* possesses properties that allow it to survive in the bloodstream.

6. Detection of *S. mutans* in heart valve and atheromatous plaque specimens

Dental caries and periodontitis are generally considered to be major diseases in the field of dentistry. Recent evidence has indicated an association of periodontitis with cardiovascular diseases [83,84], and several studies have focused on the detection of periodontitis-related bacterial species in cardiovascular specimens [85—87]. However, the oxidative condition in the bloodstream must also be considered, as it gives oral streptococci survival advantages as compared to obligate anaerobic periodontitis-related bacterial species. In a recent study, *S. mutans* DNA was most frequently detected (approximately 70%) in cardiovascular specimens from heart valves and atheromatous plaque from Japanese patients, when the presence of six oral streptococci and six periodontitis-related bacterial species was simultaneously examined (Fig. 4) [88]. A study held in the United States also reported the presence of *S. mutans* in cardiovascular specimens, though the frequency was relatively low at approximately 20% [89]. As for the serotype distribution of *S. mutans* detected in patients who have undergone cardiovascular surgery, non-*c* serotypes were identified with a significantly higher frequency in both cardiovascular and dental plaque specimens as compared

Table 3 Summary of case reports of infective endocarditis caused by *S. mutons*

Authors[a]	Country	Year	Age	Gender	Cardiac condition	Blood culture[b]	Oral condition or manipulation suspected to induce IE
Lockwood et al. [62]	USA	1974	43	F	Rheumatic disease	*S. mutans*	Tooth extraction with no prohylaxis
McGhie et al. [63]	UK	1976	55	M	IE (45 Y)/mitral regurgitation	*S. mutans* (9)	Scaling and polishing
			42	F	Heart murmur	*S. mutans*	Not described
			38	M	Mitral regurgitation	*S. mutans* (4)	Not described
Smith et al. [64]	USA	1977	41	F	Questionable history of heart murmur	*S. mutans* (10/11)	Minor dental work
Moore et al. [65]	Ireland	1977	28	M	Ventricular septal defect (repaired)	*S. mutans* (6/6)	Dental caries/ abscessed teeth
Robbins et al. [66]	USA	1977	60	F	Heart murmur	*S. mutans* (5/6)	Dental caries/ abscessed teeth
			53	M	Heart murmur due to aortic insufficiency	Negative on admission/ *S. mutans* 12 h after that	Not described
			61	M	Heart murmur	*S. mutans* (3/13)	Not described
Vose et al. [67]	USA	1987	42	F	Not described	*S. mutans* (4/4)	Filling of dental caries without prophylaxis
						→*S. mutans* (6/6)	Dental cleaning with prophylaxis
						→*S. mutans* (6/6)	Root canal treatment with prophylaxis
Ullman et al. [68]	USA	1988	69	F	Rheumatic heart disease	*S. mutans* (5/5)	Not described
			53	M	Questionable history of heart murmur	*S. mutans* (4/4)	Fractured reconstructed tooth/abscess
			56	F	Idiopathic hypertrophic subaortic stenosis	*S. mutans* (6/6)	Not described
Gauduchon et al. [69]	France	2001	63	M	Aortic stenosis	*S. mutans* (3/3)	Abscessed teeth
Nomura et al. [70]	Japan	2006	61	M	Valve replacement (59Y)	Not available	Severe dental caries

[a] Numbers in parentheses indicate reference numbers.
[b] Values in parentheses indicate the number of positive cultures. If available, the total number of blood culture examination are included. Arrows indicate recurrent IE.

to serotype *c*, which was detected in 70—75% of the samples from healthy subjects [43].

Few reports have provided a clear explanation for the association of periodontitis-related bacterial species with cardiovascular diseases in animal models, though one study showed that systemic challenge by *P. gingivalis* resulted in an acceleration of atherogenic plaque formation in a mouse model [90]. *P. gingivalis* was also shown to induce platelet aggregation and foam cell formation, both of which are known to be important steps in the development of atheromatous plaque [91,92]. It is also of interest that *S. mutans* was shown to possess these two properties [57,79,92], indicating that the bacterium is a possible candidate for inclusion in the group of bacterial species involved with atheromatous plaque formation. In addition, *S. mutans* cells bind to extracellular matrix molecules and fibrinogen [93], which is also regarded as advantageous for plaque formation. However, additional investigations with animal models are required to clarify the association between *S. mutans* and atheromatous plaque formation.

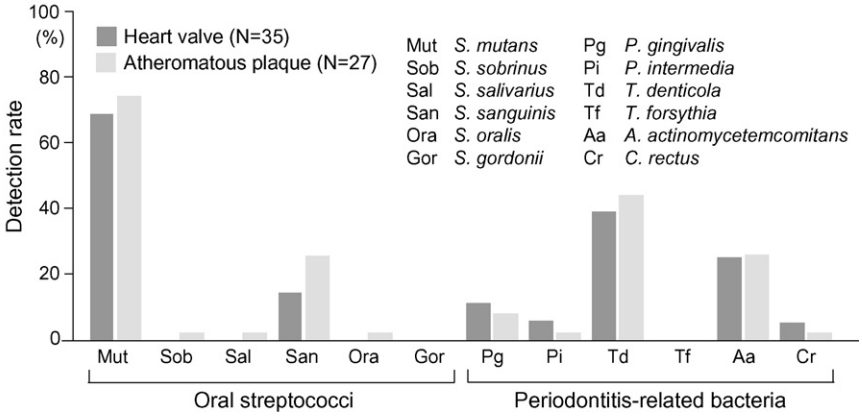

Figure 4 Detection rates of oral bacterial species in heart valve and atheromatous plaque specimens.

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